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Genetic evidence for extreme polyandry and extraordinary sex-role reversal in a pipefish

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Due to the phenomenon of male pregnancy, the fish family Syngnathidae (seahorses and pipefishes) has historically been considered an archetypal example of a group in which sexual selection should act more strongly on females than on males. However, more recent work has called into question the idea that all species with male pregnancy are sex-role reversed with respect to the intensity of sexual selection. Furthermore, no studies have formally quantified the opportunity for sexual selection in any natural breeding assemblage of pipefishes or seahorses in order to demonstrate conclusively that sexual selection acts most strongly on females. Here, we use a DNA-based study of parentage in the Gulf pipefish *Syngnathus scovelli* in order to show that sexual selection indeed acts more strongly on females than on males in this species. Moreover, the Gulf pipefish exhibits classical polyandry with the greatest asymmetry in reproductive roles (as quantified by variances in mating success) between males and females yet documented in any system. Thus, the intensity of sexual selection on females in pipefish rivals that of any other taxon yet studied.

Keywords: mating system; microsatellites; paternity; parentage; sexual selection

1. INTRODUCTION

Male pregnancy is the rule for fishes of the family Syngnathidae (pipefishes, seahorses and sea dragons). During copulation, the female transfers eggs to the ventral surface of the male, where he fertilizes and carries them until hatching (Berglund *et al.* 1986*a,b*). Trivers (1972) and Williams (1975) believed that such dramatic paternal investment should result in stronger sexual selection on females than on males in these species. However, recent work has called the generality of this prediction into question by showing that seahorses are monogamous and appear not to be sex-role reversed with respect to the direction of sexual selection (Vincent *et al.* 1992; Vincent 1994; Masonjones & Lewis 1996, 2000; Jones *et al.* 1998; Kvarnemo *et al.* 2000). Moreover, a careful examination of the syngnathid literature shows that the opportunity for sexual selection has not been quantified for any pipefish in a way that shows with certainty that sexual selection acts most strongly on females in natural populations.

Studies of sexual selection in pipefish have mainly relied on laboratory studies of mating preference (Berglund *et al.* 1986*a*, 1988; Rosenqvist 1990) or genetic mating patterns (Jones *et al.* 2000*a,b*). Although these laboratory-based studies have been very informative with respect to some important aspects of the sexual selection process, they do not provide a definitive measure of the intensity of sexual selection in nature. Two studies have examined the reproductive ecology of natural pipefish populations and provide data on their sex ratios and movement patterns throughout the breeding season (Vincent *et al.* 1994, 1995). These observations can only be translated into inferences about the nature of sexual selection when coupled with knowledge of female reproductive rates (Vincent *et al.* 1995). Such data are available and have been integrated with field studies only for *Syngnathus*

typhle (Berglund *et al.* 1988, 1989; Berglund & Rosenqvist 1990, 1993; Vincent *et al.* 1994). Observations are consistent with the idea that females experience greater sexual selection than males (Vincent *et al.* 1994), but we currently have no method for translating fluctuating operational sex ratios into a metric of sexual selection in a way that allows quantitative comparison of sexual selection among taxa.

Several other studies have described some aspects of the genetic mating systems of pipefish in nature (Jones & Avise 1997*a,b*, 2001; Jones *et al.* 1999; McCoy *et al.* 2001), but none have obtained a sufficiently complete description of the mating behaviour of both females and males for permitting a quantitative, comparative evaluation of the intensity of sexual selection. Thus, a current challenge is to obtain a sufficiently complete characterization of the natural mating system for a pipefish in order to calculate commonly used measures of the intensity of sexual selection and compare these results to similar studies of other sex-role-reversed taxa.

An excellent candidate species for additional study of genetic mating patterns and sexual selection is the Gulf pipefish *Syngnathus scovelli*. Female Gulf pipefish are larger than males and have apparent secondary sexual characters, such as a deeply keeled abdomen and brilliant stripes, which are absent in males (Brown 1972; Jones & Avise 1997*a*). These aspects of Gulf pipefish natural history indicate that the females of this species experience strong sexual selection. Our goal was to use a microsatellite-based assessment of parentage in order to test this hypothesis and quantify the magnitude of sex-role reversal for the first time, to our knowledge, in a natural population of pipefish.

2. METHODS

Gulf pipefish were collected on 28 July 1996 from a site near Titusville in the intracoastal waterway on the Atlantic coast of

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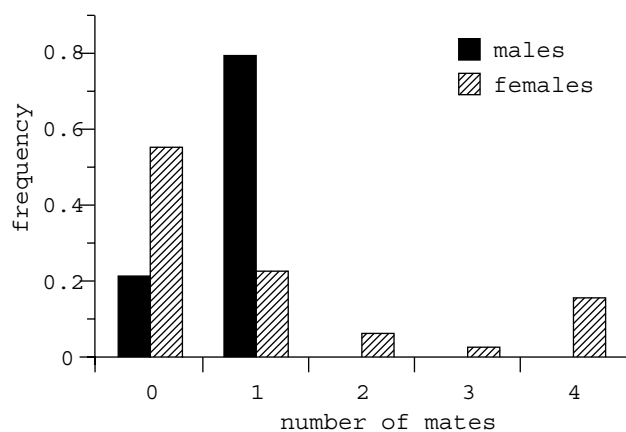


Figure 1. Distributions of the mating success of male and female Gulf pipefish.

Florida. A small patch of sea grass of *ca.* 23 m² in area and surrounded on all sides by at least 2 m of bare, sandy substrate was sampled exhaustively using a dip net. A total of 34 adult males and 27 adult females were collected, frozen on dry ice and returned to the laboratory for genetic analysis. The males and females were measured and dissected. Ornamentation was scored using an index from 1 (low intensity) to 5 (high intensity). The sexual maturity of males was confirmed by the presence of a well-developed brood pouch and that of females was confirmed by the presence of mature ova.

(a) *Microsatellite-based parentage analysis*

Twenty-seven of the 34 adult males were pregnant, 21 of which carried broods that were sufficiently developed for microsatellite assay. The broods ranged in size from six to 31 (mean of 16.4) embryos and we assayed nearly every embryo (a mean of 14 per male) using four previously described microsatellite loci (micro11.1, micro22.3, micro25.10 and micro25.22) (Jones & Avise 1997a). Polymerase chain reaction (PCR) was carried out as reported elsewhere (Jones & Avise 1997a) and the PCR fragments were separated and sized using an ABI377 automated sequencer.

Maternal genotypes were reconstructed from the microsatellite genotypes of the progeny by subtraction of the known paternal alleles. Simulations run using GERUDsim1.0 (Jones 2001) showed that both our probability of detecting multiple maternities within a brood pouch and our probability of reconstructing maternal genotypes correctly exceeded 0.99 for this particular microsatellite marker system. We used the reconstructed maternal genotypes for matching particular adult females in our collection to their offspring, which were carried in the pouches of pregnant males. We successfully identified the mothers of 14 out of the 21 broods assayed. The microsatellite markers were sufficiently polymorphic that all individuals had unique multilocus genotypes within our sample, with expected frequencies (assuming Hardy–Weinberg equilibrium) ranging from 10^{-10} to 10^{-5} . Thus, we can be certain that the genetic matches indicate the outcomes of actual mating interactions and did not occur simply by chance.

(b) *Estimation of the distribution of mating success*

Our characterization of the mating patterns in this isolated patch of sea grass was sufficiently complete to allow accurate estimation of the breeding population size and the distribution

of female mating success. The breeding population size was estimated using a mark–recapture framework (Jones & Avise 1997b) in which marked females were considered as those whose genotypes appeared in the brood pouches of the 27 males. The proportion of these genotypes appearing in our sample of adult females yields an estimate of the number of breeding females in the population (using the Lincoln–Peterson method) (Pollock *et al.* 1990) of 55.0 ± 11.2 (mean \pm s.d.). Given our estimate of the breeding population size, we used a Monte Carlo approach to obtain a maximum-likelihood estimate of the distribution of female mating success (a detailed description of this approach has been published elsewhere in Jones & Avise (1997b)). Females were constrained to mate with a maximum of four males (the maximum number of mates observed for a female in our sample), and all possible distributions with a mean mating success of unity (a consequence of the 1:1 ratio of females to pregnant males) were tested. The top 5% of simulated distributions that most often recovered our observed dataset were averaged and used as our estimate of the distribution of female mating success for this breeding assemblage. We calculated the variance in female mating success from this distribution. We ran additional simulations with differently sized breeding populations and all results were consistent with our main conclusions (e.g. the maximum-likelihood estimates of the variances in female mating success were between 1.6 and 2.1 with population sizes ranging from 35 to 75 females).

3. RESULTS AND DISCUSSION

Each embryo carried alleles consistent with paternity by the male in whose pouch it was found and each clutch exhibited a maximum of two maternal alleles per locus. Thus, each male apparently received eggs from a single female, the multilocus genotype of whom could be reconstructed by subtraction of the paternal alleles from the genotypes of the embryos. We successfully matched the adult females in our field sample to 67% of the assayed broods using these reconstructed maternal genotypes. Such a high rate of success permits an accurate estimate of the breeding population size and the distribution of female mating success (see § 2).

Our molecular results produced three lines of evidence indicating that females experience stronger sexual selection than males in this population of Gulf pipefish. First, the average number of mates per mating female was estimated to be 2.2, while the average number of mates per pregnant male was exactly unity (figure 1). Thus, their genetic mating system can be described as perfect classical polyandry, a pattern that is rare in the natural world and that is expected to produce strong sexual selection on females. Here, we use the term polyandry (Searcy & Yasukawa 1995) as a population-wide description of a mating system in which successful females frequently produce offspring with several males, whereas males sire offspring from at most one female within a well-defined breeding interval (in this case the span of a single male pregnancy).

Second, the standardized variance in female mating success (the variance divided by the square of the mean), which indicates the opportunity for sexual selection (Wade & Arnold 1980), was at least seven times higher than the standardized variance in male mating success (calculated with non-pregnant males included). This

Table 1. A comparison of the phenotypic values for females that were identified as mates of collected males ($n=7$) and females that had not mated successfully within our sample ($n=20$).

measurement	mated females		unmated females		<i>t</i> -test
	mean	s.e.	mean	s.e.	<i>p</i> -value
snout-vent length (mm)	38.10	0.70	33.80	0.90	0.01
body depth (mm)	3.80	0.20	3.20	0.10	0.02
mass (g)	0.40	0.03	0.29	0.02	0.01
ornamentation (index)	5.00	0.00	3.30	1.20	< 0.001

Table 2. Genetic mating system parameters from some of the most extremely polyandrous species ever described.

(The data for the spotted sandpiper, bronze-winged jacana, wattled jacana and red-necked phalarope came from Oring *et al.* (1991*b*, 1992), Butchart (2000), Emlen *et al.* (1998) and Reynolds (1987), respectively.)

species	number of mates per mating female	variance in female mating success	variance in male mating success
Gulf pipefish	2.27	2.03	0.17
spotted sandpiper	1.70	1.06	0.17
bronze-winged jacana	1.57	1.01	0.26
wattled jacana	1.60	0.94	0.15
red-necked phalarope	1.10	0.25	0.00

result is particularly robust given that the variance in male mating success is probably inflated, as some or all of the non-pregnant males in our collection possibly had recently given birth and were collected while they were preparing their brood pouches for a subsequent pregnancy (i.e. they may have been empty as a consequence of their reproductive cycle rather than as a consequence of competition for mates). The ratio of female-to-male variance in mating success in this population is comparable with the ratios of male to female variances observed in some highly polygynous species (Clutton-Brock 1988; MacKenzie *et al.* 1995; Searcy & Yasukawa 1995), thereby indicating that the strength of sexual selection on female pipefish may approach the intensity of sexual selection acting on some of the classic examples of dramatic sexual selection on highly polygynous males.

Finally, direct comparison of the phenotypes of the females from our sample that had mated successfully with those that were not implicated as the mates of sampled males showed that the mating females were a non-random subset of the females in the population (table 1). The mating females were larger and more ornamented than average, a result indicative of strong sexual selection on one or more of these characters (depending on the extent to which they covary). These observations are consistent with laboratory observations of other pipefish species, in which males prefer to mate with larger and more ornamented females (Berglund *et al.* 1986*b*; Rosenqvist 1993). However, to the authors' knowledge,

our results are the first empirical demonstration that these same types of characters are used as mating cues in natural populations of pipefish. Furthermore, this mating preference is so strong that it is evident in our sample, despite a small sample size. Thus, the sexual selection on females in this population must be very strong indeed. A comparison of the phenotypes of pregnant males with non-pregnant males showed no signal of sexual selection. The two groups of males did not differ in snout-vent length (*t*-test, d.f. = 10 and $p=0.89$), body depth (*t*-test, d.f. = 8 and $p=0.81$) or mass (with embryos removed) (*t*-test, d.f. = 8 and $p=0.06$).

Other species have been described as classically polyandrous, the most extreme and well-known cases of which occur in shorebirds. A comparison of the genetic mating parameters in Gulf pipefish with the mating parameters of the best characterized of shorebird taxa reveals that the Gulf pipefish exhibits the most extreme form of classical polyandry yet described (table 2). We interpret each individual's reproductive lifespan in Gulf pipefish as comprising a series of male pregnancies, each of which can be counted as a relevant period of time during which competition for mates occurs. Thus, male pregnancies are analogous to avian breeding seasons and our results can justifiably be compared with single-season studies of mating patterns in these other taxa. The mean number of mates per mating female during a single male pregnancy in Gulf pipefish is 2.27, a value substantially higher than the single-season range (1.10–1.70) (table 2) observed for shorebird populations for which the mating system has been characterized, including the spotted sandpiper *Actitis macularia* (Oring *et al.* 1991*b*), the wattled jacana *Jacana jacana* (Emlen *et al.* 1998), the bronze-winged jacana *Metopidius indicus* (Butchart 2000) and the red-necked phalarope *Phalaropus lobatus* (Reynolds 1987).

In addition, the variance in female mating success, which is directly related to the opportunity for sexual selection, is approximately twice as high in female Gulf pipefish as compared with sandpipers and jacanas (table 2). The variance in mating success is equal to the opportunity for sexual selection for perfectly polyandrous species with equal sex ratios (as both males and females must have a mean mating success of unity and the opportunity for sexual selection is the variance in mating success divided by the square of the mean) (Wade & Arnold 1980). Deviations from an equal sex ratio can affect the translation of variances into the opportunity for sexual selection, but the effect is small enough that our conclusions remain valid for a wide range of possible sex ratios. In contrast to the situation for females, successfully

mating males in all of these species mate with approximately one female and the variances in male mating success are low (table 2). The small amount of variance in male mating success that does occur is due to either males that fail to mate or the few instances of extra-pair fertilizations that have been genetically documented for some of these taxa (Oring *et al.* 1992; Emlen *et al.* 1998). In short, the Gulf pipefish appears to exhibit the most extreme form of classical polyandry yet documented, a finding that indicates that this species experiences an intensity of sexual selection on females far stronger than in any other group studied.

Although not all of the studies of shorebird mating systems discussed here used genetic markers for characterizing the mating systems, the rates of cuckoldry in these organisms appear to be sufficiently low (Oring *et al.* 1992; Delehanty *et al.* 1998; Emlen *et al.* 1998) that these intensive within-season studies of reproductive ecology probably reveal an accurate picture of the mating system and, therefore, are directly comparable with our study of the Gulf pipefish. However, studies of lifetime reproductive success (e.g. Oring *et al.* 1991a) would not be comparable and this area of research still represents a major void in the study of piscine genetic mating systems. We should also note that our study represents a single snapshot of the Gulf pipefish mating system. Temporal or spatial variation in the mating system may contribute to the dynamics of sexual selection in this species in interesting ways. Nevertheless, the mating system of this Atlantic population of Gulf pipefish appears identical to that of a previously studied population from the Gulf of Mexico, despite a striking difference in overall body size and clutch size between the two populations (Jones & Avise 1997a). Thus, all available data indicate that genetic polyandry is the prevailing mating system in this species.

Our study vindicates the views of Trivers (1972) and Williams (1975) that pregnant male fish of the family Syngnathidae provide special opportunities for understanding the nature of sexual selection. Although some species of seahorse appear not to be sex-role reversed with respect to the intensity of sexual selection (Vincent *et al.* 1992; Vincent 1994), our results show that at least some pipefish species experience very intense sexual selection on females. Thus, the family Syngnathidae provides opportunities for the study of sexual selection that are perhaps better than early researchers could have imagined. In a radiation of more than 200 species (Dawson 1985), we see examples of extreme polyandry with very intense sexual selection on females in some lineages (this study), perfect genetic monogamy with almost no apparent sexual selection in other lineages (Jones *et al.* 1998; Kvarnemo *et al.* 2000) and additional mating systems that appear to be intermediate in yet other groups (Jones & Avise 1997b; Jones *et al.* 1999). Thus, the fish family Syngnathidae represents a largely untapped resource for the study of the evolutionary causes and consequences of differences between the sexes.

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